

STUDIES UPON EPIDEMIC PARALYSIS
AMONG GUINEA PIGS

By
Louis H. Bally

Submitted to the Department of Bacteriology
and the Faculty of the Graduate School of the
University of Kansas in partial fulfillment of the
requirements for the degree of Master of Arts.

Approved by:

Submitted May 13/21

June 1, 1921.

W. H. Woodward
Department of *Bacteriology*

Acknowledgement is hereby made to Dr. Noble P. Sherwood, Head of the Department of Bacteriology, and to Cornelia M. Downs; both of whom gave their constant encouragement and many valuable suggestions in the work.

STUDIES UPON EPIDEMIC PARALYSIS AMONG GUINEA PIGS

From the Department of Bacteriology, University of Kansas

INTRODUCTION

A great many epidemics that occur among lower animals are characterized by a flaccid paralysis simulating each other in many respects. Some few cases have been reported in which the attack was very similar to acute anterior poliomyelitis in humans.

It may be stated that Römer (1911)¹ has recorded a paralytic condition, resembling poliomyelitis, in the guinea pig. He described it as an infiltrating meningo-myelo-encephalitis chiefly of the lymphocytic type. Romer attributes the infection to a filterable, ultra-microscopic, glycerin resisting virus of a similar nature to that found in human poliomyelitis.

M'Gowan and Rettie (1913)² report studies of "loupin' ill" among sheep in Scotland. This disease was characterized in the prodromal stage by extreme nervousness with a general weakening, while in the later stages a flaccid paralysis developed. They also report some cases characterized by inflammation of the respiratory passages, but seem at a loss to classify

such instances because no nervous symptoms were noticed beyond the prostration and the general weakness.

To quote one of their inferences M'Gowan and Rettie say, "As regards the pathology of this condition we believe it to be a general disease of the whole body, a particular seat of election for the action of the virus being the central nervous system. In our view, it closely corresponds in its clinical symptoms and pathological anatomy with acute poliomyelitis in man."

Two cases of paralysis among dogs are also reported. Flexner and Clark (1913)³ examined a single case which was noted to have some difficulty in swallowing. On the second day the left fore leg was lame; on the fourth day the right fore leg was completely flaccid, the head drooped and was raised with difficulty. Necropsy showed gross lesions of the spinal cord consisting of (a) perivascular infiltrations, (b) edema, infiltration and necrosis of the ground substance followed by an invasion of large phagocytic cells (compound granular corpuscles), (c) hemorrhage, and (d) necrosis of ganglion cells which became, at times, replaced by small round cells. Greeley (1917)⁴ also gives a similar report bearing the title, "An Epizootic of Poliomyelitis among Dogs". In none of these cases

was the disease attributed to any particular micro-organism.

In February, 1921 an outbreak of flaccid paralysis occurred among the guinea pigs kept by the Department of Physiology, University of Kansas. This disease was characterized by a loss of appetite, gradual decrease in weight, slight variation in temperature, emaciation, diarrhea, general tremor and indisposition to any movement except when forced from place to place. There was some variation in the length of time that these symptoms were noticeable before death, but the average time was about four or five days from the first appearance of any discomfort until death. The initial symptoms were closely followed by a flaccid paralysis of the hind limbs. The paralysis was found to occur about twenty-four hours before death. At first the animal, when urged, would attempt to move by pulling itself with the fore feet but in the more advanced paralytic stages any attempt at movement would result in the pig falling over on its side, unable to right itself.

It was the clinical resemblance of this epizootic to the disease produced in experimental poliomyelitic monkeys by Flexner, Clark and Amoss⁵ that occasioned the present work. As a typical char-

acteristic case they report: "Following an incubation period of from five to seven days there succeed excitement, general tremor, weakness, and then paralysis of the muscles affecting first the extremities, next the trunk, and lastly the respiration. Exceptionally the muscles of respiration are affected early and convulsions and death occur before paralysis of the extremities is noted."

It has been the purpose in the present paper to attempt a cultivation of the causative microorganism in this epizootic by culturing various tissues: blood, central nervous tissue and a fluid from the lungs. By inoculating brain emulsion, brain filtrate and material from the cultured virus into normal guinea pigs it was hoped that the following things might be determined:

1. What is the incubation period of the disease?
2. Does the brain or other organs of the infected pigs contain the virus?
3. Is the virus capable of passing through a Berkfeld filter?
4. Is it possible to cultivate the virus and reproduce the symptoms by inoculation after culture?

TECHNIC

In preparation for the autopsy the animal was thoroughly washed with mercuric chloride diluted 1:1000 for a disinfectant. After either shaving or plucking the hair, the animal was placed on the dissecting board ready for examination. To prevent any possible contamination two autoclaved towels were spread over the animal in such a manner as to permit the dissection without a chance for outside contamination. All instruments were autoclaved just before using.

With a pair of scissors and a sharp scalpel the skin was removed from the top portion of the head, thus laying bare the skull. This portion was again washed with mercuric chloride and the bones of the cranium were snapped with heavy bone forceps, making it possible to turn back the parietal bones and leave the cerebrum exposed. The brain was then carefully removed with sterile forceps and either preserved in 50 per cent glycerine for later use or immediately emulsified. All emulsions were made in a Rosenow sterile air chamber which had been freshly sterilized.

The material used for cultivation and inoculation experiments was brain tissue taken aseptically from either spontaneously or experimentally infected

animals. The specimens available for use were those yielded by recent autopsies and material preserved for varying periods of time. The method of preservation was to immerse the material in 50 per cent sterile glycerin and set away in the refrigerator. The material to be cultivated was taken as soon after death as possible and in no case was an autopsy performed later than twelve hours after death.

Cultivation of the organisms from the brain tissue was attempted by following as closely as possible the methods employed by Flexner and Noguchi⁶ in the cultivation of the globoid bodies of poliomyelitis. The only variation was in the protein substance used. In their work human ascitic fluid was used but as ascitic fluid was not available at this time, rabbit serum diluted 1-5 with sterile physiological salt solution was substituted.

The medium consisted of the diluted rabbit serum to which a small fragment of sterile tissue (normal rabbit kidney) had been added. Each culture was made in a deep test tube measuring 1.5 by 20 c.c. A small fragment of the sterile kidney tissue was first dropped into the tube. Next a fragment of the cerebrum of corresponding size was added. Upon these

was poured fifteen cubic centimeters of the diluted rabbit serum and finally the whole was overlaid with a four centimeter film of oil to provide anaerobic conditions. Cultures made from brain emulsions were made in the same manner, substituting one cubic centimeter of the brain emulsion for the nerve fragment. Incubation was conducted at 37.5 degrees C.

Cultures were also prepared from the heart's blood by streaking it upon blood agar plates. The blood was drawn by inserting a capillary pipette through a seared area of the heart. Two sets of plates were prepared, one to be incubated under aerobic conditions, the other for the anaerobic jar. Anaerobic conditions were provided by mixing proper amounts of pyrogalllic acid and sodium hydroxide (10 grams of pyrogalllic acid plus 100 c.c. of 10% NaOH per liter of air space) in an air tight vessel in which the plates had been placed.

The following protocols of autopsies will be used for comparison of the pathological findings in pigs dead from spontaneous infection, with those of experimental pigs.

Fig No. 1--February 21, 1921.

Virgin female; emaciation and diarrhea.

Autopsy - Lungs hardened and congested.

Small bile stained areas were noticeable throughout the liver.

Considerable gas was present in the small intestines.

Kidneys, suprarenals and spleen were normal.

The hind limbs which seemed to be completely paralysed, showed extensive hemorrhage in the groin regions. These areas extended over the fore part of the leg and to the outside.

Fig. No. 2--February 22, 1921.

The walls of the pericardial cavity were bright and glistening and a normal amount of fluid was present.

The heart was of normal size.

The left lung was distended. The right lung was collapsed. The upper left lobe was a grayish white and spongy. The lower left lobe was black and very solid on pressure.

The lung on section showed edema and emphysema; a foamy fluid oozed out of the upper left lobe.

A dark bloody fluid containing many clots was

found in the pleural cavity.

The peritoneal cavity was bright and glistening with a normal amount of fluid.

The stomach and intestines were distended with gas. The small intestines showed a yellowish discoloration.

Bile stained areas were noticeable in the liver which was pale and friable. The gall bladder was filled with yellowish bile.

The spleen was slightly enlarged and dark.

The left adrenal was slightly enlarged and the right one was somewhat reddened.

The left kidney showed cloudy swelling. The right kidney was slightly reddened; showed marked degeneration of the tissue which was spongy and readily torn. On section the cortex striations were noticeable; medulla was gray and the capsule peeled with difficulty.

Pig No. 3 - February 22, 1921.

Virgin female.

The walls of the pericardial cavity were somewhat inflamed and there was little pericardial fluid.

The heart was markedly enlarged.

The left lung was much distended. The upper

right lobe was collapsed and the lower right lobe was slightly distended. The lungs showed edema, also hyperemia. A bloody foamy fluid oozed out, when sectioned.

There was considerable gas in the stomach and intestine and the latter contained a yellowish fluid feces.

The liver was normal in size, dark red in color and spotted by the extravasation of bile.

The spleen was dark, mottled with bluish edges.

The left adrenal was slightly reddened, dark and mottled. The right was slightly enlarged.

The kidneys were red, hemorrhagic and congested. The capsule peeled readily. The interior striations were very indistinct and the medulla and cortex were not distinguishable.

There was marked hemorrhage in the groin.

Pig No. 4 - February 26, 1921.

Virgin female.

The heart was normal in size.

The pleural cavity was bright and glistening, with a normal amount of fluid.

The upper left lobe of the lung was hemorrhagic and collapsed. The lower left lobe was distended, soft, hemorrhagic and a foamy fluid oozed out. The right

lung was soft, collapsed and very hemorrhagic.

The peritoneal cavity contained a normal amount of fluid and the walls were bright and glistening.

The stomach and intestines were filled with gas, some hemorrhage being present in the intestines.

The liver was normal in size with noticeable bile extravasation.

The spleen was normal in size, somewhat spotted and dark red around the edges.

The left adrenal was slightly enlarged; the right was normal.

The kidneys appeared to be normal. The capsule peeled easily and the cortex and medulla were well defined.

There was marked hemorrhage into the tissues of the groin.

EXPERIMENTAL

For the determination of the incubation period two methods were employed, namely, contact exposure and inoculation.

Two normal pigs (male and female) were obtained from healthy stock and placed in the cage with the affected pigs, February 23, 1921. The male remained in

the cage for exposure only about ten minutes and was removed to another building. The female was left with the infected animals.

There was a gradual increase in the weight of both pigs until March 17 when the female first showed symptoms of the disease. She was extremely nervous and would not attempt to move except when forced to do so. Then instead of running naturally, she jumped after a fashion and seemed to pull herself along with the front legs, thus avoiding any use of the hind parts. Whenever the hind parts were touched, she would squeal for a considerable length of time as in pain. From March 18 until March 24th the temperature remained around 100 degrees F. By March 24 the characteristic symptoms were gradually disappearing and apparently recovery would soon occur. The control of the hind limbs had been regained and the animal moved with much more ease. The eyes seemed less blurred and the only noticeable feature was the apparent indifference to disturbance and a fatigued appearance.

During the spring recess death occurred and the carcass was lost before coming to autopsy.

This case showed an incubation period of twenty-three days.

The first symptoms of infection of the male were noticed March 21st or twenty-six days after exposure. The characteristic symptoms were just as described for the female. There seemed to be a gradual increase of nervousness and a general weakening with the development of diarrhea three days after the first symptoms. Hewas etherized March 24 and the brain removed under aseptic conditions and placed in 50% glycerine.

The question pressing at this point was whether the brain tissue was capable of setting up, in normal pigs, clinical and pathological conditions corresponding to those of natural infection. And if this was accomplished could the incubation period be more definitely determined?

Intraperitoneal and intracranial injections were made into normal pigs using an emulsion of the brain tissue and also using the filtrate obtained by passing the emulsion through a small Berkfeld filter. Intracranial injection was accomplished by slitting the skin over the forehead, then pulling the skin a little to the left and boring a very small hole through the skull, thus gaining access to the left lobe of the cerebrum. A small hypodermic needle was inserted into the opening and one tenth of a cubic centimeter of the

brain suspension was forced in. When the skin was released it slipped back to the normal position thus protecting the hole in the skull.

As a result of these experiments one out of the seven cases gave what might be termed typical symptoms. Although there was some clinical resemblance to the original cases there was not enough to warrant calling it an absolute reproduction of the disease. No paralysis of the hind parts occurred, the chief resemblances being the generally weakened condition, emaciation, loss of appetite and loss of weight. But it will be noticed that pig No. 442 lost the use of both right legs.

Protocols.

Experiment A - Male guinea pig No. 442 weight 400 grams, temperature 98.6 degrees. March 12, 1921. One tenth of a cubic centimeter of the brain emulsion from No. 3 was injected into the left cerebrum. This brain material had been preserved in glycerol for 18 days.

There was only slight variation in temperature and some decrease in weight up to April 7th when the first symptoms appeared. This gave an incubation period

of 26 days.

April 7 - Pig hesitated to move. He seemed to be in pain when movement was forced. The use of the right hind leg had been lost. Movement was very awkward.

April 8 - Right front leg seemed weakened and some difficulty was noticed in breathing.

April 13 - Slight diarrhea and tremor.

April 14 - Death.

Autopsy - There were no noticeable lesions at the point of inoculation.

The lining of the pleural cavity was bright and shining.

The right half of the lung was considerably congested, totally collapsed and a white foamy fluid oozed out when sectioned.

The stomach and intestines contained some gas.

The peritoneal cavity was bright and glistening with a normal amount of fluid.

The spleen, liver and suprarenals were normal.

The kidneys were normal in size and color, peeled easily and showed distinct divisions on section.

There was slight hemorrhage in the groin regions.

The gross appearance of the spinal cord and brain showed no lesions.

Experiment B - Male guinea pig, No. 419, weight 330 grams, temperature 101 degrees. March 12, 1921 injected intraperitoneally with .3 c.c. of brain emulsion from No. 4 which had been preserved for 14 days in glycerol..

He maintained a normal weight and temperature until March 20, when there began a rapid loss of weight.

The clinical symptoms that developed were a general weakening, emaciation and tremor but no paralytic symptoms.

March 28 - Death - 16 days after injection.

Autopsy - There were no lesions at the point of inoculation.

The pleural cavity appeared normal.

Both lungs were collapsed and edematous. Both upper lobes were blackened and hardened from hemorrhage. The lower lobes contained a foamy fluid.

The peritoneal cavity was bright and glistening, with a normal amount of fluid present.

The stomach and intestines were slightly distended with gas.

The liver was normal except for slight extravasation of bile.

The spleen was normal in size, spotted and black

around the edges.

The suprarenals were normal.

The kidneys were normal in size, slightly discolored, the capsule peeled easily and the divisions were easily distinguished on section.

There was some hemorrhage over the left groin but none in the right groin.

Experiment C - Male guinea pig, No. 422, weight 340 grams, temperature 100.2 degrees. March 12, 1921, injected intraperitoneally with .3 c.c. of a Berkfeld filtrate, from No. 4.

This specimen showed clinical symptoms very similar to those of other experimental pigs; that is, a rapid decrease in weight but almost constant temperature, emaciation and tremor during the six days preceding death which occurred April 4th or 23 days after inoculation.

The specimen was lost before coming to autopsy.

Experiment D - Female guinea pig, No. 449, weight 285 grams, temperature 103 degrees. March 19, 1921. Injected intraperitoneally with 1 c.c. of the material from an anaerobic serum media culture of brain

material from No. 4. This material was drawn with a sterile capillary pipette from the pink halo that appeared in the serum surrounding the fragments of kidney and nerve tissue. The cultural characteristics will be explained later.

No symptoms appeared until April 1, when there seemed to be a general weakening, indisposition to movement and some difficulty in breathing.

April 2 - Breathing seemed still more difficult.

April 3 - Death without manifestation of any paralytic symptoms. Death occurred 14 days after inoculation.

Autopsy - There were no lesions at the point of inoculation.

The lungs were distended and soft and very hemorrhagic. There was no foamy fluid present.

The peritoneal cavity was bright and glistening with a normal amount of fluid.

There was a bile stained fluid in the stomach and duodenum with some hemorrhage in the ileum and jejunum.

The liver, spleen and suprarenals were normal.

The kidneys were normal in size, the capsule peeled easily and the divisions showed plainly on

section. They were somewhat darkened and filled with blood.

Considerable hemorrhage was noticed in the thigh regions and blood had extravasated through large areas of the tissue.

Experiment E - Male guinea pig, No. 450, weight 275 grams, temperature 99.4 degrees. April 14, 1921, injected intraperitoneally with 1 c.c. of the brain emulsion from No. 3, which had been preserved in glycerine 51 days.

The first symptoms appeared April 30, when there had been considerable loss of weight and a sub-normal temperature. A slight difficulty in breathing was also noticeable. There was no evidence of paralysis but a general weakness and emaciation appeared accompanied by diarrhea.

May 2 - Death.

Incubation period was 16 days.

Autopsy - There were no lesions at the point of inoculation and no gross lesions of the brain or spinal cord.

The lungs were soft and collapsed. The lower lobes showed slight hemorrhage but no foamy fluid was

present.

The peritoneal walls were bright and glistening with a normal amount of fluid.

There was slight intestinal hemorrhage.

The liver and spleen were normal in size and appearance.

The suprarenals and kidneys were slightly discolored. The capsule of the kidneys peeled readily and the striations were distinct on section.

There was considerable hemorrhage in the groin regions.

Experiment F - Male guinea pig, No. 460, weight 295 grams, temperature 99.5 degrees. April 14, 1921 injected intraperitoneally with 1 c.c. of brain emulsion from No. 60. It will be recalled that No. 60 was the male that contracted the disease from contact exposure of ten minutes. This material had been preserved in glycerine 21 days.

The symptoms were identical with those of No. 450 in the preceding experiment. Death occurred May 2nd. The autopsy was very similar with the exception of a slight hemorrhage at the base of the brain and a foamy fluid was present in the lungs.

Experiment G - Male guinea pig, No. 61, weight 300 grams, temperature 100 degrees. April 14, 1921 injected intraperitoneally with 1 c.c. of Berkfeld filtrate from No. 1 which had been preserved 52 days.

There was a gradual loss of weight and some variation in temperature after April 24.

May 2 - Emaciation and indisposition to movement.

May 3 - Tremor and difficulty in breathing.

May 5 - Death.

There was an incubation period of 18 days.

Autopsy - The walls of the pleural cavity were bright and shining.

The right lung was collapsed, soft and edematous with a foamy fluid. The left lung was soft and slightly distended.

The peritoneal cavity was bright and glistening with a normal amount of fluid.

The stomach and intestines contained a bile stained fluid.

The liver, spleen and suprarenals were normal.

The kidneys were normal in size but slightly discolored. The capsule peeled easily and the divisions were plain on section.

There was considerable hemorrhage in the groin regions.

Experiment H - Male guinea pig, No. 62, weight 235 grams, temperature 101.2 degrees. April 19, 1921 injected with .5 c.c. of a suspension of facultative anaerobic, gram positive diplococci isolated from the serum cultures of the brain material from No. 449 in Experiment D.

There was a gradual wasting away and sub-normal temperature from the time of inoculation. A general weakness and emaciation were noticeable.

April 28- Death.

Autopsy - The lower lobes of the lungs were soft, collapsed, congested and edematous and a foamy fluid oozed out. The upper lobes were soft and collapsed.

The peritoneal walls were bright and glistening with a normal amount of fluid.

There was some gas in the stomach and intestines.

The liver, spleen, and suprarenals were normal.

The right kidney was normal. The left kidney was slightly discolored. Both peeled easily.

Experiment I and J - April 30, 1921, two guinea pigs (male No. 572 and female No. 761) were injected respectively with .2 cc. of serum culture from the brain of No. 1 and with 1 c.c. of brain emulsion from the pig killed with the culture of diplococci No. 62.

As yet there have been no developments from these inoculations.

BACTERIOLOGICAL FINDINGS

Sections of nervous tissue from each specimen coming to autopsy were streaked upon defibrinated blood agar plates and incubated both aerobically and anaerobically. In no case did any growth appear.

Cultures made from the heart's blood and foamy fluid of the lungs, of the first three specimens coming to autopsy, gave only aerobic spore producers. Although cultures of the organisms have been saved, they were regarded as contamination and no further investigation made. These organisms were not found in any other cases. In all other cases, the heart's blood used for culturing, either on blood agar plates or in Calcium Carbonate broth proved to be sterile.

Next, the attention was centered upon the tissue serum cultures of brain tissue. Cultures were made March 12 as described earlier under TECHNIC, using brain tissue from No. 4. Controls were made by incubating tubes of serum alone and also some with serum plus kidney.

Two days later a slight pinkish zone appeared around the kidney tissue in the inoculated tube. After six days there was a slight increase and diffusion of the pink zone, and all the tubes had a slight flocculent precipitate along the sides and gradual sedimentation. There was no noticeable change in the controls. No evidence of contamination was present in a methylene blue smear made from the cloudy area surrounding the tissue fragments.

After eight days growth some of the material from the inoculated tubes was examined under the dark field. Among the dancing protein particles were found a few small objects that resembled cocci in shape but were very much smaller. They were clumped together but seemed to be associated in pairs within the mass. One chain was noticed resembling streptococci but was so small that a focus could not be obtained, it could only be seen by focusing past it. The objects just

described were completely devoid of any movement.

Whether or not these organisms were similar to those described by Noguchi could not be determined. Although the growth characteristics and dark field appearance agreed with those of Noguchi, it was not possible to stain these organisms by Gram's or Giemsa's method.

A transfer was made from one of the characteristic tubes into the kidney control tube. After the 4th and 5th days, there appeared to be typical growth but contamination soon developed and nothing was determined.

Two pigs were also inoculated with material from these typical tubes. One died in seven days and was lost before coming to autopsy. The other No. 449 died after a period of 15 days. The autopsy for this specimen (No. 449) has been given and as will be recalled the clinical symptoms and lesions were very similar to those of cases of spontaneous infection and brain inoculations.

Other cultures of brain tissue in the serum-kidney medium invariably gave the same characteristic growth. Transfers of some of the serum were made upon plain agar slants by means of a capillary pipette. In three out of five cases growth was obtained aerobically. It was a heavy white growth, appearing in individual

colonies. Upon staining, it showed to be a Gram positive micrococci. All of the smears showed a characteristic staphylococcus grouping but within the group the appearance seemed to be that of a diplococci. In all other respects it resembled staphylococcus albus. This might be regarded as a contamination because of the omnipresence of skin cocci but the recovery of the same organism from three cultures made at different times seems to refute that. A pig was inoculated April 19, intraperitoneally with .3 c.c. of a suspension of the organisms. From the time of inoculation, there seemed to be a general weakening and emaciation accompanied by a fall in temperature and rapid loss of weight. April 27, it was found dead. The brain tissue was glycerolated and April 30 another pig was inoculated with an emulsion of the brain. There have been no developments from the inoculations.

Material drawn from near the tissue fragments of the anaerobic serum-kidney cultivations also yielded bacterial forms when streaked upon blood agar. In several cases the characteristic white growth appeared as just described. In two cases very small, clear, pin

point colonies were found. The colonies resembled those of the streptococci. When examined morphologically, they showed very peculiar shapes and arrangement.

There seemed to be some question as to whether these organisms were diphtheroids or diplobacilli. Finally they were called diplobacilli. They closely resemble the *Bacillus* of Friedlander. The growth on serum agar is very scant after 48 hours incubation. It is a gram negative, short rod, pointed at one end and the diplo form is enclosed in a capsule.

Diphtheroid Bacilli have been recovered from the lymphatic glands and mucous membranes of human poliomyelitis by Kolmer, Brown and Freese,⁷ but in no cases have diplobacilli been reported.

Nerve smears have been made in most of the cases coming to autopsy. This was accomplished by crushing a small fragment of the tissue between two No. 0 cover slips, thus making a uniform film. With a steady and quick pull, the two cover slips may be separated without destroying the smear. A special staining method devised by Noguchi⁶ was used.

After drying in the air, the cover glass is placed, film side down, in a mixture freshly prepared at each operation, consisting of one part of Grubler's

Giemsa solution, and two parts of Merck's methyl alcohol reagent, where it remains for 2 minutes.

Twenty parts of a 1 to 10,000 potassium hydrate solution are poured into the dish at the expiration of the two minutes, and the whole is thoroughly mixed by gentle agitation. At the expiration of one hour the cover glass is removed.

The film side is washed in distilled water for a few seconds after which it is differentiated in a solution of tannic acid, consisting of one or two drops of a 20% solution, added to 40 cubic centimeters of distilled water.

The cover glass is now washed in distilled water for two minutes, dried in the air and mounted.

In such film preparations organisms could be detected as being stained a deep blue against a faint blue field. Clusters of organisms were seen in several cases that resembled the clusters of staphylococcus. They were noticed to be scattered and seemed to have no particular point of localization.

In all the examinations, short chains of organisms were noticeable. These organisms were not the characteristic size of streptococci but were not small enough to be considered as globoid organisms.

These organisms were grouped in chains of four or five cells each and seemed to have a particular affinity for the nerve cells. They were almost invariably located around the edge of the cell.

Smears made from the serum-kidney anaerobic cultures were stained with Giemsa's stain. The method employed was to fix the air-dried smears in methyl alcohol for one hour with film side up. They were then transferred to a 1-10 dilution of Giemsa's stain in which they remained for eight hours. They were then washed in distilled water and mounted in balsam for examination.

Examination revealed in every case the presence of clusters of cocci that appeared to be about the size of ordinary cocci. These organisms were not very numerous and were intermingled with many clusters of very small bodies. The general appearance of these clumps was similar to that of the larger organisms mentioned but the individuals within the group were too small to permit a distinct focus upon them.

Whether these two groups belong to one and the same species cannot be said but there is certainly a great similarity. As all the films were made from seven day or older cultures, there is a possibility of a degeneration with age. Rosenow, Towne and Wheeler⁸ report

finding in poliomyelitis cocci described as short chains of diplococci resembling pneumococci, a smaller number of medium sized cocci in pairs and occasionally very small coccus forms in early cultures. After the first week, the number of small forms is relatively greater, and they have become largely gram-negative. After about three weeks large clubbed involution forms appear.

Sherwood and Downs⁹ report finding pleomorphic streptococci, culturally and morphologically similar to the organisms found by Rosenow in poliomyelitis. They were found in 20-25% of normal throats. These organisms were capable of producing similar conditions in rabbits as those from poliomyelitis and apparently have a predilection for the central nervous tissue.

Kolmer, Brown and Freese⁷ report finding a micrococcus in cerebrospinal fluid and tissues of acute anterior poliomyelitis. They describe it as a diplococcus that usually forms clumps or tetrads, grows luxuriantly on ordinary culture media, producing whitish moist colonies similar to *Staphylococcus albus*.

The growth characteristics and microscopic appearance of the diplococci isolated here seem to parallel very closely that reported by Kolmer et al.

But the author has been unable to produce a typical case by injection of a pure culture and in no instance were Kolmer and his co-workers able to produce anterior poliomyelitis in monkeys and rabbits by intracranial, intravenous, or intraperitoneal injection of these microorganisms.

DISCUSSION

Cultures made from the blood stream and the foamy fluid of the lungs gave negative results. Such findings naturally lead to the belief that the central nervous system was very probably the seat of infection. A greater stimulus to this belief came with the observation of the anaerobic serum-kidney cultures. Growth appearances very similar to those of Flexner and Noguchi⁶ were observed but with their staining methods no organisms were found that correspond to the globoid bodies. They described the typical growth of the globoid bodies as giving a faint opalescence about the fragments of tissue in the bottom of the tube, after 5 to 7 days incubation. This opalescence can be gradually diffused through the tube by gentle shaking and it is observed that the turbidity about the tissue was really greater than was first apparent. The virus

was undoubtedly cultivated because of the ability of the anaerobic serum culture to produce typical symptoms when injected.

By inoculation with brain emulsion, brain filtrate and ultramicroscopic growth, it has been possible to produce clinical symptoms and typical lesions of the lungs and hemorrhagic groin areas, very similar to those of pigs spontaneously infected. The only case in which paralysis occurred was inoculated intracerebrally with brain emulsion. The failure to produce paralysis might be attributed to an attenuation of the virus. Flexner, Clark and Amoss⁵ say, "Falling off in virulence is expressed in (a) failure to cause paralysis, (b) mild infection followed by recovery, and (c) by atypical symptoms and clinical course, followed by either recovery or delayed paralysis and death." Such common symptoms as general weakening, emaciation and tremor were produced by inoculation with brain emulsion, brain filtrate and ultramicroscopic culture growth.

M'Gowan and Rettie² describe a disease among sheep designated as poliomyelitis as having a prodromal stage in which the animal exhibits the symptoms associated with the febrile state. The temperature of the animal is raised; it does not feed; it separates from

the flock; hangs about, mopes and is listless, is constipated and has usually signs of slight respiratory catarrh. The disease is, however, declared when the nervous symptoms appear.

The disease among guinea pigs has been found to vary considerably in the length of time required for development of any symptoms. This period ranges from 15 to 26 days. In the majority of cases the incubation period was much shorter for cases resulting from injection than from cases of contact exposure.

A micrococcus has been isolated from the brain tissue by culturing in kidney-serum medium under anaerobic conditions. This organism agrees in its various characteristics to one isolated by Kolmer, Brown and Freese⁷ from the central nervous tissue of acute anterior poliomyelitis. This micrococcus is easily cultivated and grows readily on ordinary media, probably being a facultative anaerobe. A diplobacillus has also been found in two instances and is quite difficult to grow as it seems to require some body tissue.

At present there is a tendency to agree with those who consider these microorganisms as secondary and probably terminal invaders and do not credit them as the etiological agent of the disease.

SUMMARY

- I. Organ cultures (other than brain) gave negative results.
- II. Incubation varies from 15 - 26 days.
- III. An ultra-microscopic organism has been cultivated.
- IV. Two forms of facultative anaerobic bacteria have been isolated.
- V. Paralysis was produced by intracranial injection of brain emulsion.
- VI. Characteristic symptoms and lesions were produced by:
 1. Intraperitoneal injection of brain emulsion.
 2. Intraperitoneal injection of Berkfeld filtrate.
 3. Intraperitoneal injection of ultramicroscopic growth.

From these studies it will be seen that the etiological factor of the disease, is undoubtedly a filterable virus localized in the nerve tissue. This conclusion is drawn because the disease has been transmitted through two generations by the injection of emulsified brain tissue. Very similar growth characteristics to those of the globoid organisms were obtained in anaerobic serum-kidney cultures. These cultures were in turn able to produce characteristic

lesions when injected. And finally the brain filtrate also produced typical symptoms and lesions of the disease.

BIBLIOGRAPHY

1. Deutsche Med. Wochnschr. 1911, Bd. xxxvii, S 1209.
2. J. of Path. and Bact. 1913 xviii 47
3. J. Exp. Med., 1913 xvii 577
4. Med. Rec. N.Y. 1917, xcii, 839
5. J. Exp. Med. 1914, xix, 195
6. J. Exp. Med. 1913, xviii, 461
7. J. Exp. Med. 1917, xx, 789
8. J. Am. Med. Assn. 1916, lxvii, 1202
9. J. Infect. Dis. 1919, xxiv, 133